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Editorial

ATTENUATED AND MODIFIED VACCINE VIRUSES: DO THEY ACT AS A SOURCE OF SOME OTHER VIRAL DISEASES?

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ABSTRACT: For effective control of several viral diseases, live virus vaccines are given preference over killed or inactivated vaccines for their higher immunogenic potentials. Live virus vaccine strains are developed by attenuation by passaging in non-natural host species, tissues, embryonated eggs, etc., or by transferring viral genes/genomes to some other vector virus. The attenuated viruses fail to infect the species of their choice; though can elicit immunity when used as vaccines. The vector viruses are modified by the addition of a section of the genome of the disease-producing viruses in their genome to offer the desired immunity in vaccinated humans or animals. During mass vaccinations, the attenuated live viruses or vector viruses spread in the biosphere. It is suspected that many erstwhile low-profile disease agents genetically related to the viruses used in live vaccine production are causing more severe disease symptoms, changing tissue or organ of predilection, and even changing or broadening host species specificity. Three representative viral diseases affecting three species (human, goat, and cattle) are discussed as examples to enlighten the possible linkage between the spread of the attenuated or vector viruses in the biosphere and the increased virulence of related disease-producing viruses.

Key words: Live virus vaccine, Mutation, Adenovirus, COVID-19 vaccine, Lumpy Skin Disease, Goat Pox vaccine, Peste des Petits Ruminants, Rinderpest Vaccine.

Vaccination is an indispensable tool to induce specific immunity against specific pathogens in an active and acquired manner. Another form of active immunity development happens following natural means - by clinical or subclinical infections followed by recovery.

In contemporary vaccinology, control of infective viral diseases is mainly performed by vaccination. The vaccine types available to prevent viral diseases are live-attenuated vaccines, inactivated vaccines, replicating and non-replicating viral vector vaccines, DNA vaccines, RNA vaccines, recombinant vaccines, etc. (Tezard 2018, Dai *et al.* 2019).

As the killed or inactivated virus vaccines are less efficient to confer strong and long-lasting immunity, the attenuated live disease-causing viruses or other viruses with segments of their genome became the main techniques of vaccine production. So, live vaccines constitute the bulk of viral vaccines used in humans and animals. Live vaccines are made of avirulent strains of a disease-causing agent: either naturally avirulent or less virulent strains are used or virulent strains are made avirulent through passage/ forced replication in unnatural hosts such as unnatural host species, tissue culture, or embryonated eggs.

Like other fields of science, the technologies for vaccine development are changing fast, as we see in the use of vector-vaccines or RNA vaccines against the SARS-CoV-2 virus during the COVID-19 pandemic. Live virus vaccines are preferred over killed vaccines because of their replication in the host body and thus higher immunogenic ability. However, the use of live viruses or their genetic materials in vaccination may also cause the spread of those viruses or their genetic materials in the biosphere which may undergo mutation or recombination. There are some suspicions of the occurrence of new viral diseases, an increase in the pathogenicity of some lowly pathogenic viruses, a change in the host specificity of the viruses, etc. pointing to the use of live vaccines. Few such suspected examples are put forward to get some direction towards prevention of such problems in the future.

A. ADENOVIRUS INFECTION OF CHILDREN AND COVID – 19 VACCINES

Adenovirus infection is generally limited to mild flu-like symptoms among healthy individuals but may cause severe illnesses among individuals with lower immunity status or having pre-existing cardiac or respiratory

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diseases (CDC 2022). But in recent times, particularly during the late COVID-19 pandemic years, kids up to five years of age having no previous record of any serious or co-existing insufficiencies are suffering from symptoms of severe breathing difficulties to acute hepatitis leading to death in some cases after getting an infection of adenovirus (https://www.thelancet.com 2022, Wang *et al.* 2022, https://www.thehealthsite.com 2023). In the UK, such acute hepatitis in children is suspected to be due to infection by adenovirus subtype 41, though the subtype was previously known to be linked only with mild to moderate gastrointestinal symptoms (https://www.thelancet.com 2022). Death of children due to adenovirus infection has been reported from many parts of the world covering almost all continents of the globe (Baker *et al.* 2022, Gong *et al.* 2022, https://www.thelancet.com 2022).

The sudden increase in virulence, change of affected organs and systems, and more vulnerability of children to the virus need the attention of the researchers.

There are few plausible explanations for such instances such as conspiracy of disease creation, sequelae of COVID-19 vaccination, infection of adeno-associated virus 2 with helper virus co-infection, etc. Some other hypotheses are the immunity deficit among children due to lack of exposure to pathogens during the COVID-19 lockdown period, relaxation of lockdown caused a massive wave of adenovirus, past infection or co-infection, exposure to certain toxins or drugs, environmental factors that changed immunity to the virus among children, etc. (https://www.thelancet.com 2022). However, the affected children probably were not vaccinated against the COVID-19 virus (since they are up to 5 years of age) and the adenovirus infection with higher virulence is not confined to a single country (Baker *et al.* 2022, https://www.thelancet.com 2022, Gong *et al.* 2022), and as such the raised hypothesis are perhaps not supported by sufficient logic.

Adenovirus is used as a vector for the preparation of vaccines

Adenoviruses are non-enveloped, medium-sized, double-stranded DNA viruses with icosahedral nucleocapsid. Adenoviruses are having the ability to drive expression of the target antigen among the vaccinated entities strongly, and so it is preferred for use as a vector for other candidate vaccine viruses. Over 150 adenoviruses have been characterized from different primate species, mostly to assess their capability for use as vaccine vectors. The virus is used as a vector in the preparation of vaccines against Acquired Immuno-Deficiency Syndrome (AIDS), Zika virus disease, Ebola virus disease, malaria, and tuberculosis. For COVID-19 vaccine preparation, the human (adenovirus 5 and adenovirus 26) and chimpanzee serotypes were mainly tried. In adenovirus vectored vaccine preparation, generally, the E1 and E3 viral genes are deleted and replaced with the transgene of interest for the expression of desired antigen(s). COVID-19 vaccines such as Ad26.COV2-S by Janssen/Johnson & Johnson, ChAdOX1-nCoV by Oxford/AstraZeneca, Ad5-nCOV by CanSino Biological Inc, Gam-COVIDVac/ Sputnik V by the Gamaleya Research Institute, etc. are prepared using adenovirus as vector (Mendonça *et al.* 2021).

Adenoviral diseases of animals

Adenoviruses can infect several vertebrate species like birds, reptiles, bats, fish, amphibians, etc. The important adenoviral diseases of the animals that stay close to humans are Infectious Canine Hepatitis (ICH) and Kennel cough in dogs, adenoviral respiratory infections in horses, cattle, pigs, sheep, and goats, etc. (Harrach *et al.* 2019). But there is no evidence of human infection from these animal adenoviruses so far (Sykes 2014).

Is there any mutation of the adenovirus infecting children?

Adenoviruses have been used as vectors in many previous vaccines created to control diseases like AIDS, Ebola, or Zika virus diseases. But such vaccinations were produced on much smaller scales and used in a limited number of individuals. None of those vaccines have been used at a large scale like the COVID-19 vaccines and different human and chimpanzee strains of adenoviruses have been used for making COVID-19 vaccines (Jacob-Dolan and Barouch 2022). This enhances the chance of the spread of the adenovirus gene and/or its modified genome among humans, animals as well as in the virus population in recent times.

Children below five years of age are having much less susceptibility to suffering from COVID-19 and have a chance of getting any other vaccine with an adenovirus carrier (https://www.thelancet.com 2022). So, possibly they did not have sufficient antibodies against adenoviruses or their modified genomes used in different vaccine preparations which might make them vulnerable to infection of any genetically modified virulent adenovirus. This also raises suspicion of any relation between the spread of genetically modified adenoviruses or their modified genes in the biosphere (maybe by the related Covid-19 vaccines) and the change in virulence

and organ or system specificity of the adenoviruses infecting kids!

B. LUMPY SKIN DISEASE AND GOAT/ SHEEP POX VACCINES

Lumpy skin disease (LSD) is a disease of cattle caused by a Poxvirus, a double-stranded DNA virus. The main symptoms of the disease are fever, the formation of large nodules in the skin over the neck and other body parts as well as on the mucous membrane of internal organs like digestive and respiratory tracts (Mulatu and Feyisa 2018). The causative agent of LSD belongs to the genus Capripoxvirus that also includes Sheep pox virus (SPV) and Goat pox virus (GPV) (Barrett and McFadden 2008); these viruses share many genes that lead to the idea that they have originated from a common ancestor. LSD virus genes and proteins have also a high degree of similarity with many other viruses of the same family, like Suipoxvirus, Leporipoxvirus, and Yatapoxvirus (Tulman *et al.* 2002).

The vaccines prepared from sheep pox or goat pox viruses are partially protective against LSD in cattle (Coetzer and Tustin 2004, Hamdi *et al.* 2020).

Is there any relation between SPV or GPV with increased incidence and death by LSD?

As per the old reports, the morbidity of LSD generally varies between 2 - 45% and the mortality is generally less than 10 %. The susceptibility to the disease is dependent on the immunity status and breed of the animal; the European breeds are more susceptible than the breeds from Africa and Asia (Tuppurainen *et al.* 2017). But in the recent LSD outbreaks in India, a much higher rate of morbidity and mortality of infected cattle are found.

Among a huge number of infected cattle, more than 1.65 lakh cattle died in a few months in India (https://www.thehindu.com, https://www.outlookindia.com, Down to earth 2023).

The goat pox or sheep pox vaccines used routinely are all live-attenuated vaccines and are used for the vaccination of a large number of sheep and goats every year. LSD virus is generally not used to vaccinate cattle. Goat or sheeppox viruses are genetically very closely related to LSD viruses. There are some minute differences in the genomes as well as in the surface antigens of these three viruses (sheep pox, goat pox, LSD). But LSD infection has been noticed among the cattle vaccinated with goat pox virus recently (Down to earth 2023).

Due to the rapid spread of the 'attenuated' strains of the live viruses in the animal population as well as in the biosphere following mass vaccination, there was an increased scope of mutation in the genome of any of them.

Suspicion arises, is it the modified strain of LSD that broke the resistance of the cattle and caused havoc loss in India with the recent LSD outbreak?

C. PESTE DES PETITS RUMINANTS (PPR) AND RINDERPEST VACCINES

Peste des petits ruminants (PPR) is a disease of sheep, goats, camel, and many other wild ruminants. It is caused by a member of the genus Morbillivirus of the family Paramyxoviridae, an enveloped virus with a non-segmented negative-strand RNA genome. The PPR virus is having only one serotype, but it can be differentiated into four lineages by nucleic acid sequencing (WOAH 2020). PPR is characterized by acute onset of fever and anorexia, followed by serous nasal discharge, necrotic stomatitis, conjunctivitis, respiratory distress, severe diarrhea, emaciation, dehydration, dyspnoea, and death within 5-10 days. The disease can cause a huge loss of the susceptible population by its very high morbidity (90-100%) and mortality rates (50-100%). The disease is transmitted by aerosols, direct contact, and via fomites from the affected animals to the susceptible. So, nasal discharge, tears, cough, and all other secretions and excretions of the animals during the incubation period as well as during the diseased condition contain infective viruses (Golchinfar *et al.* 2011, Balamurugan *et al.* 2014, WOAH 2020).

Interrelations between PPR and Rinderpest viruses

The PPR virus of caprine, the Rinderpest virus of cattle, and the Measles virus of humans possibly diverged from a common precursor (Tezzard 2021). The Canine Distemper virus is also having antigenic similarities with these viruses (WOAH 2020). Very close similarities are found between the PPR and Rinderpest virus genome, antigenicity, as well as in disease symptoms. An incubation period of a few days followed by depression, anorexia, and development of ulcers in the mucous membranes leading to respiratory distress, diarrhea, and death are the common symptoms found in both of them (Wohlsein and Saliki 2006, Golchinfar *et al.* 2011, Tezzard 2021). The vaccines prepared by milder strains of PPR viruses to control the PPR of goats can also partially inhibit the Rinderpest virus in cattle, but only vaccination by wild type of PPR virus can protect cattle from the Rinderpest virus challenge (Holzer *et al.* 2016).

One study found that after vaccination by PPR vaccines, the antibodies cross-react with live Rinderpest virus, and the reverse reaction was also observed

Table 1. Examples from three different species for possible correlation with initiation/ potentiation of other viral diseases by use of live virus as some vaccine item.

Disease with increased virulence	Main symptoms	Previous history of the virus	Contemporary concepts	Related vaccine	Similarity, between the viruses	Possible reason/s and alike disease/s in other animals
Adenovirus infection of children	Acute hepatitis and respiratory distress	Caused mild flu like symptoms	Conspiracy of disease creation, sequelae of COVID-19 vaccination, infection of Adeno associated virus 2 with helper virus co-infection, lack of exposure to pathogens in lockdown period, relaxation of lockdown caused massive wave of Adenovirus, past infection or co-infection, exposure to certain toxins or drugs, environmental factors, etc.	COVID-19 Vaccines (Vector vaccine)	Modified genome of related strain used directly	Vaccine strains may act as modifying agent of any prevailing strain after reaching to the ecosystems.
Lumpy Skin Disease (LSD) of cattle	Fever, enlarged lymph nodes under skin and other body parts, mucous membrane of internal organs, very high morbidity and mortality rate	Can cause similar type of diseases among sheep, goat	A separate disease than sheep pox or goat pox.	Goat pox vaccine (live, attenuated)	Antigenically close, partially cross - protective	Vaccine strains act with other strains or assist to mutate to become more infective and pathogenic after reaching the biosphere
Peste des Petits Ruminants (PPR) of goat and sheep	Acute onset of fever, anorexia, followed by serous nasal discharge, necrotic stomatitis, conjunctivitis, respiratory distress, severe diarrhoea, emaciation, dehydration, dyspnoea and death within 5-10 days	Similar type of lesions were found in Rinderpest of cattle, presently declared as eradicated.	A separate disease, not related with Rinderpest vaccine	Rinderpest Vaccine (Goat adopted, rabbit adopted, bovine kidney cell cultured live vaccines)	Antigenically close, partially cross - protective	PPR and Rinderpest are having same ancestor; any of the goat adopted or other type of live viruses used in vaccines may caused mutation and increase in infectivity of the PPR virus.

(Couacy-Hymann *et al.* 2006). So, these two viruses are very close in consideration of antigenicity, genome structure, disease symptoms, etc.

Vaccines used for more than fifty years to control Rinderpest

For vaccine development, the virulent Rinderpest virus was injected into the living goats for successive generations. By such serial passage inside the non-natural host of the Rinderpest virus (non-cattle host), the virulence of the Rinderpest virus was reduced. Then the 'caprinized' strain of Rinderpest virus was injected into cattle as some attenuated live virus vaccine. The process continued up

to the 1950s. The virulent Rinderpest virus was similarly passaged in living rabbits for 600 generations to get the 'lapinized' strain of Rinderpest virus to use as a live, attenuated vaccine for cattle. These two types of vaccines (caprinised and lapinized) were used in cattle up to the 1960s. Afterward, the Rinderpest virus was cultured in bovine kidney cells up to at least 95 subcultures before use as tissue culture attenuated live viral vaccine (Tezzard 2021).

Rinderpest disease of cattle is already declared irradiated by vaccination in 2011 after ceasing vaccination totally from 1997 in India (NPRSM 1997) and 2006 from the globe (Tezzard 2021).

Why PPR is so violent?

Almost all strains of the PPR virus are highly virulent. No mild strain of the PPR virus is identified (Wohlsein and Saliki 2006). Previously, the disease was not so common. The magnitude of PPR has increased only in the recent past (NABC 2013).

Initially, the Rinderpest virus was passaged into living goats, then into living rabbits for generations. Then the tissue culture attenuated live virus vaccines came to the field for use. Vaccination against Rinderpest continued for a few decades. If we consider the time frame, along with widespread vaccination and the resultant reduction of cases of Rinderpest among cattle and afterward emergence of highly pathogenic strains of PPR have emerged! This raises suspicion whether the spread of the vaccine virus in the biosphere has led to an increase in its virulence and/or change in species specificity over time.

D. HORIZONTAL GENE TRANSFER AMONG VIRUSES: ROLE OF HOST AND VIRUSES

There are proofs of the transfer of part of the viral genome into the host cell (Desfarges and Ciuffi 2012) and also the transfer of the host genome into viruses (Caprari *et al*. 2015, Weiss 2017). There are also identified evidences of horizontal transfer of part of the viral genome into other related as well as unrelated viruses (Caprari *et al.* 2015, Koonin and Krupovic 2022). In such a condition, the assumption of horizontal transfer of viral gene/genome to related viruses getting a fair chance of interaction and formation of more potent viruses with changed species specificity cannot be considered as pure hypothetical assumptions.

During mass vaccination, both in humans and animals, the spread of the genome of vaccine viruses, carrier viruses, as well as the modified genomes themselves, get ample chance to spread in the host population as well as in the entire biosphere (Caprari *et al*. 2015, Makarenkov *et al.* 2021). Along with related or unrelated pathogenic viruses, many non-pathogenic viruses living in our immediate environment and the biosphere can come into their contact. The mixing vessel effect can contribute to the development of more pathogenic viruses or conversion of non-pathogenic viruses to pathogenic ones or donating the power to change the species specificity towards any non-natural species of animals or humans cannot be ruled out easily. Examples of three such important diseases infecting three different species and causing huge loss and harm in recent years in different parts of the world are also displayed in a tabular form (Table 1).

CONCLUSION

The practice of vaccination with modified live viruses, their relatives, or by genetic engineering of any virus to get desired protection level in humans and animals has got tremendous momentum in recent years. But these processes may have some other sides also. Three examples of such live vaccines and suspected cases of change of virulence and species specificity of other related viruses are analyzed to highlight some of the problem areas. Time has come to understand these problems and searching out of any adjunct or alternate way to overcome them.

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